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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/031,241	01/17/2002	Bernhard Hauer	50531	6324
26474	7590	01/25/2006	EXAMINER	
NOVAK DRUCE DELUCA & QUIGG, LLP			PAK, YONG D	
1300 EYE STREET NW			ART UNIT	
SUITE 400 EAST			PAPER NUMBER	
WASHINGTON, DC 20005			1652	

DATE MAILED: 01/25/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/031,241

Applicant(s)

HAUER ET AL.

Examiner

Yong D. Pak

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 7/20/2005.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-22 is/are pending in the application.
- 4a) Of the above claim(s) 1-10,13-15 and 19-22 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 11,12 and 16-18 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Claims 1-22 are pending. Claims 1-10, 13-15 and 19-22 are withdrawn. Claims 11-12 and 16-18 are under consideration.

Response to Arguments

In view of the appeal brief filed on July 20, 2005, PROSECUTION IS HEREBY REOPENED. New grounds of objection are set forth below.

To avoid abandonment of the application, appellant must exercise one of the following two options:

- (1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,
- (2) request reinstatement of the appeal.

If reinstatement of the appeal is requested, such request must be accompanied by a supplemental appeal brief, but no new amendments, affidavits (37 CFR 1.130, 1.131 or 1.132) or other evidence are permitted. See 37 CFR 1.193(b)(2).

Claim Objections

Claim 11 is objected to because said claims depend from non-elected claim.

Claim 16 is objected to because "zing" should be "zinc".

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 11-12 and claims 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 11-12 recite the term "hydroxylatable". A fatty acid that has a capability of being hydroxylated conveys that the fatty acid is hydroxylated under some conditions but may not be hydroxylated under all or other conditions. Therefore, it is not clear what

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are those conditions in which the fatty acids has the "capability" of being hydroxylated.

Examiner requests clarification of the above phrase and suggests deleting said phrase.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 11-12 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 11-12 and 16-18 are drawn to a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids using cytochrome P450 monooxygenase and electron donor system comprising a non-electrode-bound source of electrons and a mediator. These claims are drawn to a method of enzymatic production of any or all terminally or subterminally hydroxylated fatty acids using any or all cytochrome P450 monooxygenase, including any recombinants, variants and mutants from any source, and/or any electron donor system. Therefore, the claims are drawn to a method for enzymatic production of a genus of terminally or subterminally hydroxylated fatty acids using a genus of cytochrome P450 monooxygenase and a genus of electron donor systems. There is insufficient descriptive support for the genus comprising any or all cytochrome P450 monooxygenases, genus comprising any or all hydroxylated fatty acids and genus comprising any or all electron donor system. The specification only teaches a method of hydroxylating fatty acids described in Examples 2-4 of the specification using a cytochrome P450 monooxygenase obtained from *Bacillus megaterium* and zinc/Co(III)sepulchrate. These examples are not enough and does not constitute a representative number of all the species to describe a method of hydroxylating a genus of any or all fatty acids using a genus of cytochrome P450 monooxygenase, including any or all mutants, variants and recombinants, and/or genus of any or all electron donor systems. Further, there is no evidence on the record of the

relationship between the structure of the fatty acids described in Examples 2-4 of the specification and the structure of any or all fatty acids, which is a large class of chemical compounds. On similar lines, there is also no evidence on the record of the relationship between the structure of a *Bacillus megaterium* cytochrome P450 monooxygenase and the structure of a polynucleotide encoding any or all any recombinants, variants and mutants of any cytochrome P450 monooxygenase nor a relationship between the structure of a zinc/Co(III)sepulchrate electron donor system and the structure of any or all electron donor systems. Therefore, the specification fails to describe a representative species of a genus of cytochrome P450 monooxygenase and genus of electron donor systems used in a method of hydroxylating a genus of fatty acids.

Given this lack of description of the representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the inventions of claims 11-12 and 16-18.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov <<http://www.uspto.gov>>.

Claims 11-12 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of hydroxylating fatty acids described in Examples 2-4 of the specification using a cytochrome P450 monooxygenase obtained from *Bacillus megaterium* and zinc/Co(III)sepulchrate, does not reasonably provide enablement for such a method of hydroxylating any fatty acids using any or all variants, mutants and recombinants of any or all cytochrome P450 monooxygenase and any or all electron donor systems. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of

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direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 11-12 and 16-18 are drawn to a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids using cytochrome P450 monooxygenase and electron donor system comprising a non-electrode-bound source of electrons and a mediator. These claims are drawn to a method of enzymatic production of any or all terminally or subterminally hydroxylated fatty acids using any or all cytochrome P450 monooxygenase, including any recombinants, variants and mutants from any source, and/or any electron donor system. Therefore, the claims are drawn to a method of hydroxylating fatty acids having any structure using any or all recombinants, variants and mutants of any cytochrome P450 monooxygenase having any structure and electron donor systems having any structure.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of fatty acids, any or all variants, mutants and recombinants of any cytochrome P450 monooxygenase and/or any or all electron donor systems broadly encompassed in the method of the claims. Regarding cytochrome P450 monooxygenases, since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The claims also encompass a method of introducing double bonds into any position within a steroid skeleton using any bacterial host cell.

However, in this case the disclosure is limited to a method of hydroxylating fatty acids described in Examples 2-4 of the specification using a cytochrome P450 monooxygenase obtained from *Bacillus megaterium* and zinc/Co(III)sephulchrate, but

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provides no guidance with regard to hydroxylating any or all fatty acids using any or all cytochrome P450 monooxygenase, including variants, mutant and recombinants, and/or any or all electron donor systems. It would require undue experimentation of the skilled artisan to make hydroxylated fatty acids as claimed. In view of the great breadth of the claim, amount of experimentation required to identify and make the necessary cytochrome monooxygenase and electron donor system, amount of experimentation required to hydroxylate any or all fatty acids, the lack of guidance, working examples, and/or unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the monooxygenase and electron donor systems to hydroxylate any or all fatty acids as encompassed by the claims.

While enzyme isolation techniques, recombinant and mutagenesis techniques and other related techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications in a polypeptide as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method of hydroxylating any fatty acids using any or all cytochrome P450 monooxygenase from any source and any electron donor system because the specification does not establish: (A) a universal method to terminally and subterminally hydroxylate any or all fatty acids; (B) regions of a cytochrome P450 monooxygenase structure which may be modified without affecting specific dehydrogenase activity; (C) the general tolerance of cytochrome P450 monooxygenase to modification and extent of such tolerance; (D) a rational and predictable scheme for selecting any fatty acids ,

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cytochrome P450 monooxygenase and electron donor system with an expectation of terminally or subterminally hydroxylating any fatty acids; and (E) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including method of using any fatty acids, any variant, mutant or recombinant of any cytochrome P450 monooxygenase and/or any electron donor system to hydroxylate said fatty acid. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of cytochrome P450 monooxygenase having the desired biological characteristics and electron donor systems having the desired biological characteristics to hydroxylate any fatty acids is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 11-12 and 17-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Estabrook et al.

Claims 11-12 and 17-18 are drawn to a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids comprising hydroxylating fatty acids in the presence of an electro donor system, a cytochrome P450 monooxygenase, oxygen, chloride ions and a hydrogen peroxide-cleaving enzyme, wherein said fatty acid is a C-12 fatty acid and wherein said electron donor system comprises an inorganic, non-electrode-bound source of electrons and a mediator.

Estabrook et al. (*Methods in Enzymology* – form PTO-1449) discloses a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids comprising hydroxylating fatty acids in the presence of an electron donor system, a cytochrome P450 monooxygenase, oxygen, chloride ions and a hydrogen peroxide-cleaving enzyme, wherein said fatty acid is a C-12 fatty acid and wherein said electron donor system comprises an inorganic, non-electrode-bound source of electrons and a mediator (pages 45-46). Therefore, the reference of Estabrook et al. anticipates claims 11-12 and 17-18.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claim 16 rejected under 35 U.S.C. 103(a) as being unpatentable over Estabrook et al. in view of Creaser et al.

Claim 16 is drawn to a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids comprising hydroxylating fatty acids in the presence of an electro donor system, a cytochrome P450 monooxygenase, and oxygen, wherein said electron donor system is zinc/Co(III)sepulchrate.

Estabrook et al. discloses a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids comprising hydroxylating fatty acids in the presence of an electro donor system, a cytochrome P450 monooxygenase, and oxygen, wherein said electron donor system comprises an inorganic, non-electrode-bound source of electrons and a mediator, as discussed above. The method of Estabrook et al. uses a Co(III)sepulchrate mediator of Creaser et al. because “it retains chirality during reversible oxidation-reduction” (page 45, 1st paragraph).

Creaser et al. (J. Am. Chem. Soc – form 1449) discloses a Zn/Co(III)sepulchrate electron donor system, which pioneered for the use of Co(III)sepulchrate as mediators

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in electrochemical reactions (Faulkner et al., Reipa et al. – US Patent 6,126,795 and Roberts et al. – US Patent 6,492,132). Creaser et al. teaches that Zn dust causes reduction of the Co(III)sepulchrate mediator within seconds (page 3181).

Therefore, in combining the teachings of Estabrook et al. and Creaser et al., it would have been obvious to one having ordinary skill in the art to use either Zn dust as originally taught by Creaser et al. or Pt in the method taught by Estabrook et al. One of ordinary skill in the art would have been motivated to use Zn dust because Creaser et al. teaches that Zn dust causes immediate reduction and because Zn dust is widely available (Sigma). One of ordinary skill in the art would have had a reasonable expectation of success since Estabrook et al. teaches a method of hydroxylating fatty acids with cytochrome P450 monooxygenases by replacing NADPH with an electrochemically generated reduction by the mediator Co(III)sepulchrate and Creaser et al. teaches a method of generating two electrons using the mediator Co(III)sepulchrate and Zn dust as the source of electrons.

Therefore, the above references render claim 16 *prima facie* obvious to one of ordinary skill in the art.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants also argue use of improper hindsight reasoning. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In the instant case, it should be noted that the knowledge of a Zn/Co(III)sepulchrate electron donor system was well known and within the level of one having ordinary skill in the art at the time the invention was made. With such information already available in the prior art, one having ordinary skill in the art would have been motivated to use Zn dust as a source of electrons.

Applicants argue that Creaser et al. does not teach or suggest applying Zn/Co(III)sepulchrate electron donor system in enzyme-catalyzed hydroxylation reactions of fatty acids and the suggested treatment of said system does not represent physiological conditions application to enzyme-catalyzed reactions. It appears that applicant's arguments are against the references individually. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The reference of Creaser et al. is relied on its teaching of using Zn dust as the source of electrons. Estabrook et al. provides disclosure of using an electron donor system comprising a Co(III)sepulchrate as the mediator in enzyme-catalyzed hydroxylation reactions of fatty acids. In combining the teachings of Estabrook et al. and Creaser et al., it would have been obvious to one having ordinary skill in the art to use either Zn dust as originally taught by Creaser et al. or Pt in the method taught by Estabrook et al. One of ordinary skill in the art would have been motivated to use Zn dust because Creaser et al. teaches that Zn dust causes immediate reduction and because Zn dust is widely available (Sigma).

Applicants also argue that the electron donor system of the present invention is higher in reaction rates when compared to the electron donor system of Estabrook et al. Again, it appears that applicant's arguments are against the references individually. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. The relative rates of the electron donor system for Estabrook et al. and Zn/Co(III)sepulchrate electron donor system is irrelevant because the rejection is based on the combined teachings of Estabrook et al. and Creaser et al. Further, Examiner notes that the claims do not recite any such limitations on the rate of reaction.

None of the claims are allowable.

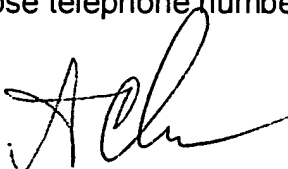
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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak
Patent Examiner 1652



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